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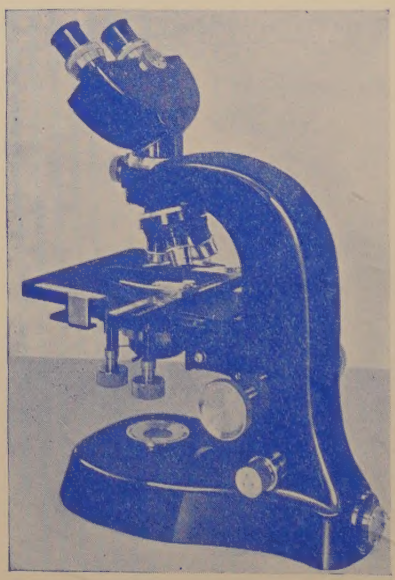

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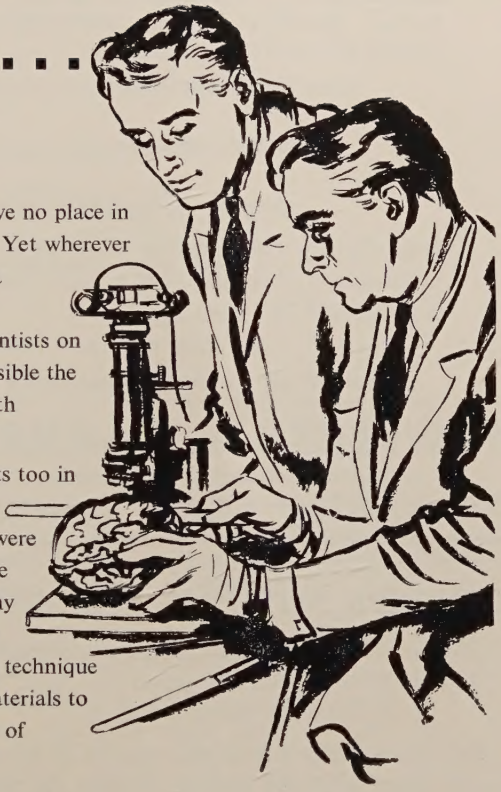

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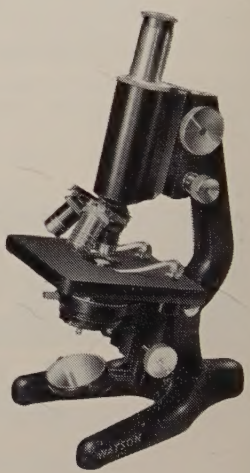

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THE MICROSCOPE

The British Journal of Microscopy and Photomicrography incorporating "The Entomological Monthly"

Arthur L. E. Barron, FRMS, ARPS, Editor

January - February, 1960
Vol. 12 No. 6

EDITORIAL

WITH the publication of this first issue for 1960 we are happy to say that the major production difficulties which we encountered over the past year are now behind us. We take this opportunity of thanking our readers, and our advertisers as well, for having borne with us during this difficult time; especially gratifying were the many letters of encouragement received in response to the circular sent out last December. This letter, explaining the continued delay in publication, was sent to all direct subscribers and as many more readers as we could reach by post or through distributors.

This year we shall be publishing a new 'How To' series of articles. This series will not follow a rigid pattern — as, for example, a purely beginners' series — but will be varied in both subject and content, in order to appeal to the widest possible audience. Newcomers to microscopy in particular, who would like any special subject dealt with, are invited to send in their suggestions. In planning the programme we have had to look ahead and therefore it may not be possible to give immediate effect to a specific request, but we do assure you that the suggestions we receive are always most carefully considered.

THE MICROSCOPE can only reflect the interests of its readers and we can only continue to give you what you want while readers are interested enough to write for us. If you feel you cannot manage a full length paper or article, a short note or a letter, recording an observation, describing a new piece of apparatus or a modification to an existing one, an improvement to a method of preparation, or a short cut, will be equally welcome. Likewise, if you have any problem do not fail to write to us, if we do not know the answer, we will publish your letter.

HOW TO STUDY NANNOPLANKTON

E. G. WILLIAMS, M.A., F.R.I.C.

In 1911 Lohmann published a paper on the nannoplankton and its collection in the living state by the centrifuge[1] and D. J. Scourfield also published a paper on the 'Use of the Centrifuge in Pond-life Work'[2].

Scourfield, whom I had the pleasure and advantage to know, also wrote in *Watson's Microscope Record*[3] on this subject and his article gave me my first introduction to this very interesting study. Pond-life had been a hobby of mine since school-boy days but I never seemed to get very far with systematic work. However, the nannoplankton article gave me a new impetus and I obtained a small hand centrifuge with special lightweight head and tube, and as I use a high-power binocular I had to manage tube-length correction of apochromatic objectives by means of the Sir Herbert Jackson Aplanatiser supplied by Messrs. W. Watson & Sons Ltd.

Optical equipment

The microscope objectives selected were 16 mm ($\frac{2}{3}$ in.), 8 mm (Watson's apo N.A. 0·65) and a 4 mm Zeiss apo N.A. 0·95. In addition I had available a Zeiss J water immersion with correction collar, and a Watson's 'Holos' 2 mm, N.A. 1·37. I found that with × 10 eyepieces (Swift's Telaugic) on my Watson's 'Bactil' high-power binocular and the extra length of the Aplanatiser I obtained a magnification of about 300 with the 8 mm objective and about 600 with the 4 mm objective and that this was a much as I could usefully use for most of the work.

I had studied A. C. Coles's 'Critical Microscopy' and for some years deliberately used a $\frac{1}{2}$ inch wick paraffin lamp (in spite of the smell !).

For checking the optical set-up I used a Hyrax mounted slide of *Surirella gemma*. The substage condenser was a Watson's 'Universal' with sufficient aplanatic cone for the 4 mm objective.

I have gone into some detail in regard to apparatus for I believe that, unless sufficient attention is paid to technique, not only will work suffer but the investigator will become dissatisfied and give up. I wrote an article for *Watson's Microscope Record*[4]

in which I stressed that observational technique is very important, we are dealing with extremely delicate structures the refractive index of which differs only slightly from that of the medium in which they are observed. Note that phase contrast techniques had not become generally known or available in 1934. It might be profitable for someone possessing the equipment to work over the field of nannoplankton with phase contrast methods.

Nature and occurrence

Now as to the nature and occurrence of nannoplankton. The word *nanno* comes from the Greek *nanos* meaning dwarf (German *-swerg*) (I don't know how it came by its two n's), and of course *plankton* is also Greek meaning wandering and is applied to those forms of life which float passively or have a limited amount of locomotion.

In " British Freshwater Algae " [5] West and Fritsch say that the comparative richness of any district in freshwater algae depends largely on its physical geography and geological formation, but the study of the nannoplankton seems to have given this somewhat old-fashioned (1904-1927) view quite a new outlook. For the development of this theme reference should be made to Professor F. E. Fritsch's Presidential Address to the British Ecological Society, 10th January, 1931[6]. It so happened that the writer of these notes started his study of Algae (nannoplankton) in a lowland district amongst the waters of the Cheshire Plain around Chester and was lucky enough to find a very rich and abundant nannoplankton in waters which hitherto had not been deemed rich. The late D. J. Scourfield gave invaluable help, and with the aid of various publications such as the " Susswasserflora Deutschlands, Osterreich U. der Sweiz " [7], " Wisconsin Survey Bulletins " [8] and " Freshwater Algae of the United States " [9] the writer was able to go forward with the indentification of forms hitherto classed as rarely found. Furthermore, the facility enjoyed in the ability to sample continuously throughout the year enabled the various maxima of forms to be noted.

The results obtained by sampling in various places from the Island of Mull in Scotland to the Lynns of North Wales as well as the Cheshire lowlands were published in the *North Western Naturalist*[10] during the years 1933 to 1948, and in the *Proceedings* of the Chester Society of Natural Science, Literature and Art in 1949, 1951 and 1954[11].

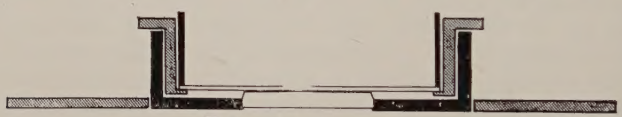
Reference should be made to the work of B. M. Griffiths on the phytoplankton of various bodies of freshwater from the Norfolk Broads to Anglesey and North Wales[12]. This does not exhaust the bibliography by any means, and I have no doubt the full references up to date would be supplied by the Freshwater Biological Association of the British Empire at Windermere. The

writer feels that wide reference and adequate reading are essential to this subject which offers opportunity for fascinating and original research.

Collection and Examination

The above introduction seems to call for the recipe for jugged hare — first catch your hare ! — and so we must turn to methods of collection and examination. Since the finest bolting cloth has openings amounting to 100μ and even the finest silk gauze, according to D. J. Scourfield, has openings of 50μ, it is no use depending on them to sieve out organisms of 25μ or less, hence the value of the centrifuge. My own hand-driven centrifuge with lightweight head, gives an RCF (relative centrifugal force) of about 1272 at about 4,000 revs per minute and containing two tubes of about 1·8 mls capacity. An electrically driven centrifuge of small type gives an RCF of about 1840 with six tubes each containing 15 mls, or with 2 × 15 mls tubes, an RCF of about 2366. The RCF of 1272 is quite sufficient for general work on the nannoplankton organisms, but the convenience of the electically operated machine, as well as the much greater volume of fluid dealt with, is appreciable.

Having obtained the small amount of sediment at the ends of the tubes the question of its transference to the microscope for viewing arises. With a fine pipette of suitable capacity the top liquid should be taken off without disturbing the sediment, quite an easy job with ordinary care. Sufficient liquid should be allowed to remain in the tube to allow the sediment to be stirred into a turbid ' broth ' by means of a fine bore pipette and squirting action, and one or two drops transferred to the slide. This may be a live box, of the Rousselet type, or even a coverglass supported



Rousselet live box.

by a ring of Vaseline on an ordinary slide. But the coverglass should be thin enough for highpower work and there should be adequate room for the sometimes bulky nose of the objective to cover the field right up to the edge. Also it is necessary to control evaporation as observation may be prolonged.

What will be visible on looking down the ocular will depend largely upon the source of the nannoplankton. At certain times of the year it may well be that organisms will be few and at other times there tend to be too many for comfortable scrutiny. The writer's own work has shown well defined maxima and minima in

various types of nannoplankton of small bodies of water, as well as of larger bodies such as lakes and rivers. This, of course, has been well observed and reported by many observers in time past in connection with the plankton more usually gathered by means of the net.

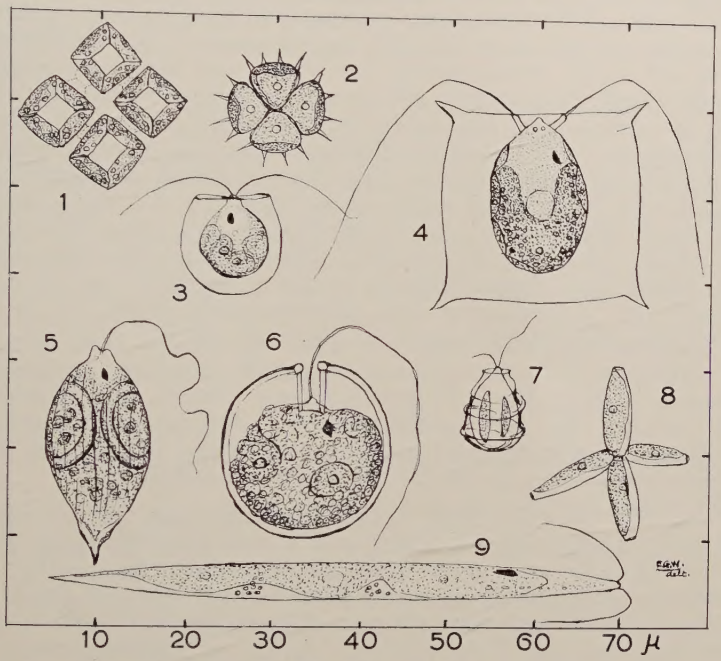


Fig. 1. *Crucigenia fenestrata* Schmidle. CHLOROCOCCALES.
Fig. 2. *Tetrastrum staurogeniaeform* (Schroder) Lemm. CHLOROCOCCALES.
Fig. 3. *Pteromonas angulosa* (Carter) Lemm. VOLVOCALES.
Fig. 4. *Pteromonas aculeata* Lemm. VOLVOCALES.
Fig. 5. *Lepocinclis steinii* var. *suecica* Lemm. EUGLENINAE.
Fig. 6. *Trachelomonas volvocina* var. *cervicula* (Stokes) Lemm. EUGLENINAE.
Fig. 7. *Pseudokephyrion* sp. CHRYSOMONADINAE.
Fig. 8. *Actinastrum hantzschii* Lagerh. CHLOROCOCCALES.
Fig. 9. *Chlorogonium* (? *elongatum*) Dangeard. VOLVOCALES.

The drawings are semi-diagramatic with conventional "shading". It is not possible to reproduce adequately the delicate structures as seen under the microscope. The Chlorococcales usually have pale to bright green chromatophores, the Volvocales bright green chromatophores with bright red "eye-spot", the Eugleninae have bright dark green or yellowish green chromatophores with bright red "eye-spot". The Chrysophyceae have delicate brown, olive brown, orange or yellowish orange chromatophores and red "eye-spot" (where present).

The organisms

Now as to the nannoplankton organisms themselves, they are of very wide distribution. Any water showing a distinct turbidity of

a greenish hue will yield an abundance of the Volvocales, Chlorococcales, Eugleninae or all mixed. Sometimes a fairly pure gathering of one species may be obtained but more generally the sample will contain an abundant mixed phytoplankton, often abounding in forms classed as rare, with a sprinkling of new forms awaiting description. Summer and autumn months tend to show maximum growth, especially of certain groups; but interesting forms are to be found during the winter and especially spring months, chiefly those with brown, orange or yellow chromatophores such as *Mallomonas, Dinobryon* spp. *Uroglena volvox* and other Chrysophyceae. Planktonic diatoms such as *Stephanodiscus hantzschii* and *Cyclotella* spp. may often be found in abundance, but Desmids, in lowland waters at any rate, are not likely to be abundant. Members of the Cryptophyceae are often abundant and offer a good field for research. Myxophyceae are often well represented (at times forming water 'blooms') species of *Microcystis, Aphanocapsa* and *Merismopedia* being of usual occurrence, and *Anabaena* spp. are also met with.

Members of the Dinophyceae are usually to be found, often in abundance. Eugleninae are sometimes met with in great quantity and variety. As well as the coloured Protophyta, colourless flagellates are also frequent either free-swimming or epiphytic on diatom frustules or threads of the filamentous algae.

In 1933 to 1935 small bodies of water near Chester were regularly sampled by the writer and a rich nannoplankton was discovered. Let me take from my notebook the results of gatherings made during one year from a small body of water in the Eaton Hall Estates near Chester. Eleven species of Volvocales were identified and 79 species and 'forms' of Chlorococcales, 8 species of Chrysophyceae, 8 species of Eugleninae, 8 species of Myxophyceae and smaller numbers of species of Heterokontae, Cryptophyceae, Conjugateae and Dinophyceae. There were members of the Diatomaceae, mainly forms of *Asterionella, Stephanodiscus, Cyclotella, Navicula* and especially a 'find' in *Attheya zaccharíasi* Brun., a diatom described from Continental waters and the only freshwater representative of the marine family Biddulphoideae.

The Chlorococcales showed a marked summer maximum and a great variety of species. Chrysophyceae as well as the Diatomaceae tended to show a spring maximum and there was a general reduction or disappearance of all forms during the months of November, December and January.

The particular reaction to temperature and sunlight of any piece of water must not be taken for granted but must be worked out by direct observation over a series of samples at regular and short intervals,

Bottom-living forms

In addition to the surface-loving forms there are also forms which exist in the darker and de-oxygenated layers of the bottoms of ponds, and here we meet with various bacteria and bacteria-like forms. There are to be encountered the sulphur bacteria such as *Thiopedia* and *Beggiatoa* and the motile *Purpur* bacteria. There are also a variety of small and ' difficult ' organisms of considerable interest.

It will be difficult, at first, to ' see the wood for the trees ' and the writer has found it advantageous to commence by selecting some of the non-motile forms for observation. Accurate drawing is greatly to be desired but some form of diagram is essential, especially as a first-time quick note. More deliberate attention can be made at subsequent observations. Frequently the ' little-by-little ' technique will have to be employed until the observer has become acquainted with the form. It is this first strangeness which is such an obstacle, but with patience it can be overcome, and the whole field opened up more and more to reveal its great and absorbing interest.

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This is only a small portion of the bibliography which will, of course, grow as reading progresses.

THE USE OF PHOTOMETERS FOR DETERMINATION OF EXPOSURE TIME IN PHOTOMICROGRAPHY

Part 3

K. A. REYNOLDS

THE reader will have already observed that as the exposure time determining procedure described in the preceding articles entails measuring the image intensities for *particular selected tones* of the scene, the photometer used to make the measurements must be capable of measuring the intensities of *small selected areas* of the image. It is to the construction and use of an instrument of this type that subsequent articles will be devoted, but in view of the fact that a high proportion of those who have applied photometers to photomicrography have used not selective instruments but integrating ones, which measure the *average* intensity of the image as a whole just as an ordinary photoelectric exposure meter measures the average brightness of the scene as a whole, some consideration may first be given to this kind of measurement and its limitations.

Average image intensity

Given an image occupying the emulsion plane and filling the frame which delimits the emulsion area to be exposed, a very convenient method of obtaining an integrated intensity measurement of that image is to place in the emulsion plane a photovoltaic cell sufficiently large to occupy the whole of the frame and so receive exactly the same image on its sensitive surface as will the emulsion. The reading shown by the meter to which the cell is connected represents the total quantity of light falling on the cell surface per unit time, and so, provided the area of that surface remains constant, represents also the average intensity of illumination of that surface. For this latter to be the same as average image

intensity, the cell surface must be fully occupied by image. Where it is necessary to use a cell of smaller size than the image the average measured is of course that of the portion of the image which falls on the cell, which may or may not be representative

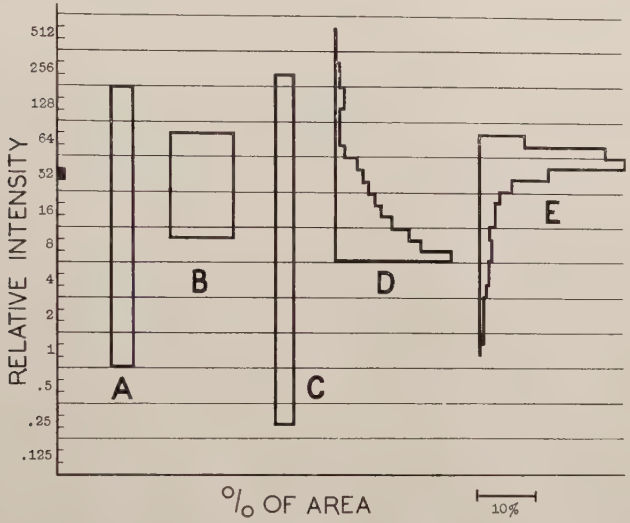


Fig. 1. Percentages of area occupied by the different intensities (measured to nearest third-of-a-stop) in five different imaginary images all having the same average intensity of 32 and so giving the same reading from an integrating meter.

of the whole. Photocells are sometimes used in less direct ways, at planes other than that of the emulsion (cf. Part 1, p. 69), but these ways will not be discussed here.

Given this measure of average image intensity we now have to ask whether it constitutes adequate information about the image for the purposes of exposure time determination. Unfortunately the answer is that by itself it does not. We have already seen that the key information for use in exposure time determination is maximum and minimum intensity, but these two quantities bear no fixed relationship to the average intensity and so cannot be deduced from it. The flexibility of the relationship between extremes and average is illustrated in Fig. 1, which consists of histograms showing percentage of image area occupied by each of the intensities present in a number of imaginary images all having the same average intensity. In A, B, and C, all the intensities included in the range are present occupying equal percentages of the image area, but in D and E, which are more realistic cases, inequality of the percentages of the image area occupied by the different intensities is also introduced, showing how the spacing of the

extremes relative to the average can be affected by preponderance of one part of the range in the particular field of view chosen.

To take a specific example of this latter effect consider a scene consisting of densely pigmented hairs under the microscope, transilluminated on a bright field, and suppose that the light transmitted to the eye — or emulsion — by the substance of the hair is only 6% of that transmitted by adjacent clear mount, and that the field is occupied by several hairs covering 55% of its area. An integrated measurement of the intensity of the image of this field will then be 45 × 100 + 55 × 6 units, i.e. 4830 units. Now suppose that the slide is moved to obtain a field which includes only one hair, occupying 5% of the area: by thus bringing into a further 50% of the field the full transmission of the clear mount the integrated reading is increased to 95 × 100 + 5 ×6, i.e. 9530 units, which is almost twice the first reading. In spite of this change in the reading the second field still requires exactly the same exposure time as the first, for the maximum and minimum intensities have not changed. More extreme examples than this can readily be imagined, involving intensity range as well as relative quantities of area occupied.

The 'average scene'

It may well be asked at this point: why is it then that integrated scene brightness measurements are so much used, and with so much apparent success, in ordinary photographic practice? The use of integrated brightness measurements in ordinary practice is based on the concept of the 'average scene'. Thus the scene taken as 'average' in the British and in the American standards for photoelectric exposure meters has a brightness range of 1:128, which, with the various tones occupying equal areas of the scene, gives an average brightness of 32 (Fig. 1, A), and the assumption is that a high proportion of the scenes met with in ordinary photography have a relationship between average and extremes not greatly different from this. The 'camera exposure' (i.e. the shutter time and lens aperture combination, which might better be termed the light admission setting of the camera) recommended for use in conjunction with the reading given by a meter measuring average scene brightness is such as to yield, in black-and-white negative making, a somewhat greater than optimum — i.e. than minimum useful — exposure from this 'average' scene. It is greater than optimum in order that there shall be some safety margin to take care of scenes which depart somewhat from 'average' in having a greater separation between average and minimum brightness. If the scene departs widely from 'average', an intelligent correction of the light admission setting of the camera must be applied, or the photographer allows himself to be badly misled by the meter.

The practical success of this concept of the 'average scene' thus depends on the great majority of scenes dealt with in ordinary photography being of generally similar nature — usually consisting of numerous and various objects all illuminated in the same manner and seen by the light they more or less diffusely reflect — and on the practicability of characterising the main departures of scenes from 'average' and providing suitable correction factors for them. In other words, the average brightness is not used alone, but is heavily supported by a more or less valid pre-assumption as to its approximate relationship to the extremes. The success also depends heavily on the fact that in black-and-white negative making much of the error that does occur through failure to make proper allowance for scenes which depart from 'average' is accommodated by the exposure latitude of the negative-yielding process, which can allow the use of a generous safety margin. This is not the case with reversal and other processes which lack latitude however, and that is why the average brightness measurement is giving way to more selective measurements among users of such processes.

In photomicrography the variety of commonly met with scenes is if anything greater than in ordinary photography, so that to use average image intensity measurements with any success it is essential to take a number of scenes each 'average' for its particular limited class of object and illumination and percentage of area occupied by different tones, for instance an 'average' stained thin tissue section transilluminated on a bright field and filling the field, or an 'average' etched metal surface seen by reflected light and filling the field, or an 'average' diatom transilluminated on a bright field of which it darkens a negligible area, and so on. For each of these classes a table must be drawn up, on the basis of practical trials, showing optimum exposure times for various processes against average image intensity, and each scene must be assigned to its appropriate class before the exposure time can be looked up. Such a system may serve adequately enough for the worker who is concerned with only a few classes of scene, but it is troublesome to establish and somewhat cumbersome to use for the photomicrographer whose work may range over the whole variety of possible classes, i.e. all the many possible combinations of tone range, tone preponderance, and rendering-requirements: for him the simple versatility of the procedure described in Part 2, capable of dealing with any type of scene without preamble, but involving selective measurement, is much to be preferred.

Selective measurement by means of integrating meters

Most scenes consist of small intermingled areas of various tones, and without selectively measuring them there is no knowing which

of them corresponds to the average as measured by the integrating meter. If however the ' scene ' consists of a uniform expanse of a single tone, then the average is the same as the actual tone. Thus in this situation the integrating meter becomes equivalent to a selective meter. The only requirement is that the whole field covered by the meter be occupied by the one tone. While there are many occasions in photomicrographic work where it is not practicable to obtain a uniform expanse of a relevant tone such as to fill the whole field, there are two very important classes of work where this can usually be achieved, and where the integrating meter can in fact be used for the procedure set out in Part 2 just as well as a meter which will selectively measure small areas of the field.

The first of these classes of work is that where opaque objects are photographed by the light they reflect. The procedure here for the photomicrographer (equally valuable to the general photographer) is to first choose the field he wishes to photograph, then select the tone to be rendered as white, then seek, in some part of the object itself or from some external source such as a set of suitable white and light grey surfaces, a large enough area of that tone to fill the field for the purpose of taking a measurement. The measurement taken, the field to be photographed is moved back into position and the exposure made. Similarly with the other class of work which is that employing ordinary bright field transillumination, and in which the required area of uniform highlight tone is almost always available as some part of the mount in which slide, mountant, and cover are present just as in the field to be photographed, but no object is present.

In theory, measurements from the darkest tone to be rendered, such as the object free background tone in darkground types of illumination, may be employed in the corresponding way, and this would be preferable when using black-and-white negative-yielding processes, but in practice the meter may not always be capable of measuring the low intensities involved.

When it is necessary to use a highlight image intensity measurement in conjunction with a black-and-white negative-yielding process the aim is to place the highlight at a point on the useful density range such that the shadows will also be accommodated. The simplest way to ensure this is to place the highlight at the maximum useful density of the process, so that all scenes of which the range is equal to or shorter than that of the process are thereby accommodated. With scenes which have a range smaller than that of the process however, this results in giving more than the minimum useful exposure, which is undesirable practice. It is therefore best to avoid this by using a simple scene classification into say long, medium, and short range scenes, and placing the highlights of these at progressively lower points on the density range as illustrated in Fig. 2.

It is of course possible to make a photoelectric image intensity meter selective simply by reducing the surface area of the cell, so that only a small portion of the image can fall on it, and this

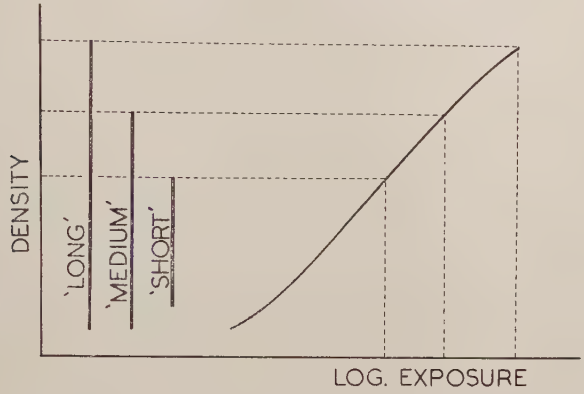


Fig. 2. Illustrating the principle of placing highlights of scenes of different range at different points on the useful density range of a black-and-white negative-yielding material.

small portion can be a selected uniform area of the tone chosen for measurement. But in order that illumination of such a small area of cell surface may produce a readable needle deflection, it becomes necessary to use a suitable amplifier between cell and meter, and the apparatus thus becomes more elaborate and costly.

Summary

The making of integrated image intensity measurements does not itself constitute an adequate basis for exposure time determinations, but needs to be supported by a scene classification embodying adequate limitation of each class with respect to tone range, tone preponderance, and nature of required rendering. The necessity for drawing up such a detailed classification, and of drawing up from the results of trials a separate exposure-time table for each class, is avoided by using the previously explained method of working from selective image intensity measurements. There are important circumstances — namely those where the whole image area can be filled by a uniform relevant tone — in which integrating meters (most of which are of the photoelectric type) can be used in effect as selective meters, and can then be relied on to yield exposure time determinations of consistent high accuracy.

Commercially available equipment (cf. Part 1, p. 69)

In Britain interest has recently been shown in the commercial manufacture of integrating photoelectric exposure meters intended

specifically for photomicrography. Evans Electroselenium (see *B. J. Phot.* Vol. 105, 1958, p. 314, and Vol. 106, 1959, p. 172) and Cooke Troughton & Simms (*B. J. Phot.* Vol. 106, p. 172; *The Microscope* Vol. 12, p. 79) have been developing prototype instruments. A number of continental, and of American, firms are now advertising equipment of this kind, the meter in some cases being built in, together with the camera, as a combined " photomicroscope " stand. Some of this equipment receives mention in the latest books on photomicrography, thus to mention two examples, Needham in " The Practical Use of the Microscope," 1958, gives some account of the use of the American Photovolt meters, and Heunert in " Praxis der Mikrophotographie," 2nd Ed. 1959, of the Zeiss eyepiece camera with built-in meter. But for information of this kind of equipment it is mostly necessary to search advertisement and equipment review pages in those various medical, scientific, and technical journals which devote some attention to microscopy.

TIN AND ITS USES - SUMMER 1959

THIS issue of ' Tin and Its Uses ' describes several new projects involving tin.

Tin has always been considered harmful in cast iron but recent careful investigations show that, properly controlled, it suppresses ferrite, and promotes the formation of the pearlite constituent without tending to cause massive cementite. This may lead to better wear-resistance in cast iron, coupled with good machineability.

A new flux medium, polyethylene glycol, reduces the bad spattering effects which cause the corrosion encountered with ' killed spirit ' (zinc chloride). The new fluxes spread much better and more uniformly and help solder to penetrate further into joints.

Wood-destroying fungi were unable to attack specimens of wood which had been impregnated with certain organotin compounds. Details of trials carried out at Delft, Holland, indicate that one of these compounds is particularly promising as a preservative.

1960 is the 150th anniversary of the invention of tinned foods and plans for celebrating the event are being prepared in many countries. In this issue of ' Tin and Its Uses ' a synopsis of the plans so far announced indicates that England and the United States both regard the occasion as being of the highest importance.

SOME PROBLEMS OF COLOUR PHOTOMICROGRAPHY

K. E. GIBSON, F.R.M.S.

Reciprocity failure

It is more than half a century since Abney showed that the photographic emulsion does not obey the simple reciprocity law of Bunsen and Roscoe and that the developable effect produced by a certain exposure depends not only on the product of exposure duration and illumination intensity but on their actual respective values.

Either by accident or design, photographic materials show maximum sensitivity when illumination intensity is such as to give 'correct' image density at about $^1/_{10}$th second exposure duration.

Deviations from this optimum exposure duration result in a reduction of emulsion speed and is responsible for many under-exposed negatives and transparencies, the exposure for which may have been most carefully calculated.

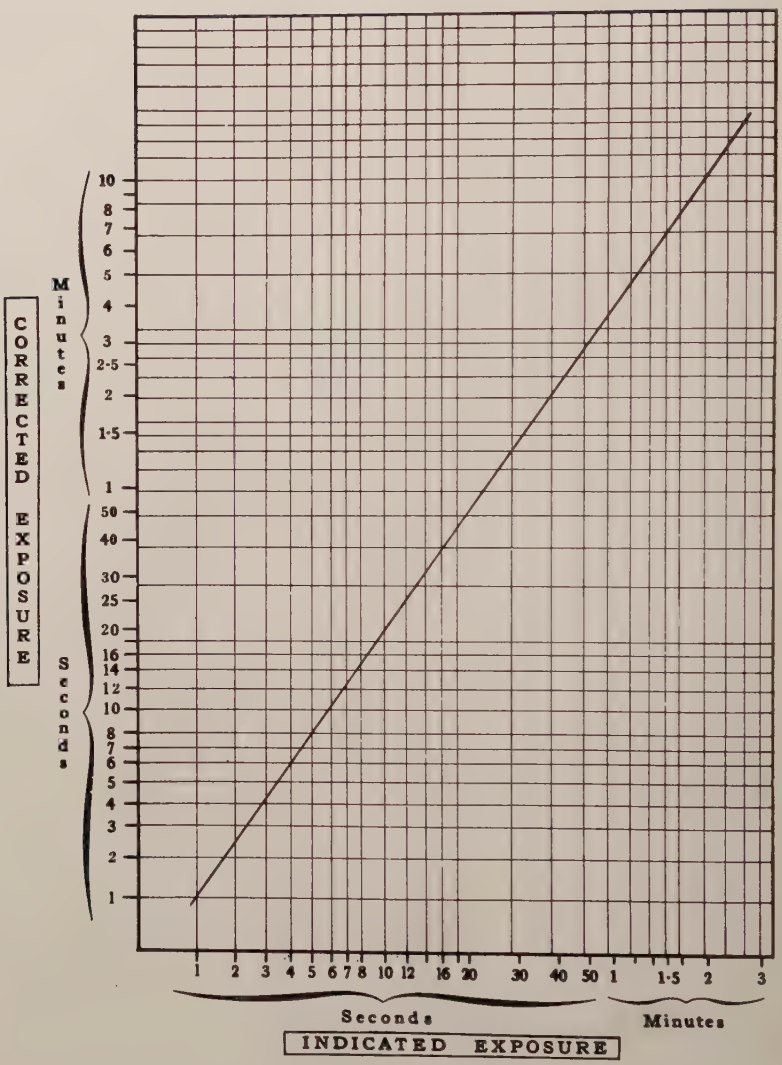
Monochrome negative materials show a certain degree of latitude when used on normal photomicrographic subjects and the operator, when assessing an exposure of long duration can 'add a bit' for luck and stand a fair chance of success. If, however, the exposure has been wildly inaccurate, the cost of a retake is negligible.

With colour materials on the other hand, no latitude is available and the cost of materials coupled with the long delay between exposure and viewing of the result, usually precludes any possibility of a retake.

For these reasons, the author conducted a series of tests, which resulted in the exposure correction curve illustrated. The film used to determine the curve was Kodak Ektachrome E 2, Type F.

At the time of writing, the curve has been in use for about a year with great success. Although produced for the film mentioned, some Ektachrome E 1, Type B has also been used with the curve, the results from which suggest that the curve can be used with colour materials of different manufacture.

Another effect of reciprocity failure that needs to be mentioned is the upsetting of colour balance due to the three colour-sensitive



Curve illustrating the correction for reciprocity failure of colour film. Although determined for Kodak Ektachrome E2, type F, it is suggested that the graph may be equally suitable for other materials.

layers of the film suffering from reciprocity failure to differing degrees.

For most work the author uses Ektachrome Type F material, which is so manufactured as to give correct colour balance when exposed for $^1/_{50}$th second (the effective duration of a flash bulb). When this material is used with a 3200°K. ribbon filament lamp, some 'primary' filtering of the illuminant must be used to bring the colour temperature up to the 3800°K. of the normal white flash bulb, for which the film is balanced.

Further primary filtering to correct for the green cast produced by the glass elements of microscope optics, must also be incorporated.

It follows, that during tests to ascertain the correct primary filtration required, if the exposure duration remains constant at about 2 seconds, then correction for reciprocity failure unbalance at this exposure must automatically be incorporated in this primary filtration.

In the author's experience, increase of the exposure duration in excess of two seconds brings about no appreciable amount of further colour unbalance, and any that may occur is lost among the many other factors that tend to upset colour balance.

Mains voltage fluctuations

In the author's laboratory, the illumination from a mains voltage lamp was read at intervals with a colour temperature meter. At midday, when maximum load was on the local sub-station, the colour temperature was 2225°K., at 8.00 p.m. it was 2475°K., a difference of 250°K. which is enough to produced marked differences in colour balance of pictures taken at these times.

Obviously, if some degree of consistency in results is required, a means of regulating the voltage supplied to the illuminant is necessary. This can be achieved by one of three methods:—

(a) By fitting a commercially produced constant voltage unit. These are very much a 'fit-and-forget' instrument, having no movable parts they never require attention but like all good things, are very expensive, and on this score, can only be recommended to the laboratory with a large quantity output.

(b) by using a toroidally wound variable transformer of the 'Troidac', 'Variac' or 'Regavolt' type, in parallel with a voltmeter. The voltage to the lamp (or step-down transformer, in the case of a low voltage lamp), can be set manually before each picture is taken.

(c) by using a large rheostat and voltmeter in the same way as (b) above. Commercially produced units working on this system can be purchased from photographic suppliers but all suffer from the one disadvantage, that a voltage lower than the

input voltage must be selected to feed the lamp and consequently reduces the brilliance of the lamp.

Ageing of the lamp

From the moment a lamp is put into service its colour temperature steadily drops until very near the end of its life, when the colour temperature again rises. The drop can be as much as 300°K., which is quite considerable when one realises that the human eye can detect differences of 100°K. with ease. Only by checking results from a test slide and applying filtration can uniform results be obtained.

Fading of Filters

Under the action of heat and light, all filters (with the exception of those dyed in the melt) lose their colour so slowly and insidiously that the fading is not noticed, unless a recent photograph is compared with an earlier one, or regular use is made of a test slide.

Yellowing of balsam mounts

Natural resin mounts have the unfortunate habit of becoming yellow with age, and whilst one accepts as normal a balsam slide which is slightly discoloured, a colour photomicrograph of that slide, in which the background is anything but water white, is viewed with some concern.

To obtain an approximate correction for mountant colour, lay the slide on a viewing box and match the colour of the mountant with colour filters of the Kodak C.C. series or equivalents, then place over the microscope light source filters that are complementary to the 'matching' filters, for example, a slide photographed recently matched the combination C.C. 10 Y, C.C. 05 Y and C.C. 05 R. The filters used to obtain a neutral background were, C.C. 10 B, C.C. 05 B and C.C. 05 C.

Processing variations

Before attempting to produce the reciprocity failure correction curve, a test was made to assess the consistancy of the processing service offered by professional processing houses. Identical films were sent to two well known firms and one film was processed by the author. The difference between the three results was most remarkable! Not only was colour balance vastly different but effective film speed varied by as much as 200%.

Needless to say, the author strongly recommends 'home' processing for qualitative and quantitative colour photomicrography!

Batch variations

Although manufacturers of colour materials still recommend testing each new batch of material before attempting serious work, in practice the little variation that is found is of no great consequence.

Test Slide

As an aid to consistent results, a test slide can be easily made by laying very thin strips of gelatine filters, etc., on a slide and covering with a cover glass held down with strips of adhesive tape. No mounting medium should be used and the slide will resist fading if kept by itself in a ' single ' slide box.

If the test slide is photographed at intervals, it will be a useful check on processing and film batch qualities, etc. Should the operator wish to assess a new make of film or check some out-dated stock, the test slide will be found invaluable, providing that a full record of objective, condenser, ocular, filters, exposure duration and magnification is made and repeated in subsequent tests.

REPLIES TO CORRESPONDENTS

A reader in Cape Town asks for information concerning the preparation of sponge spicules and sections of sea urchin spines —

Cleaning sponge spicules

Most sponges have siliceous spicules. The animal substance may be destroyed by treating it with strong nitric acid or a mixture of strong nitric and sulphuric acids. It is preferable to do this without the application of heat, unless time is important. Spicules of calcium carbonate occur only in a few sponges, e.g. *Grantia:* they may be recovered by macerating in a strong solution of sodium hydroxide. The spicules should be freed from acid and detritus by successive washings and decantations through clean water, finishing with distilled water, and then stored in distilled water to which a few drops of formalin have been added. If, as may well be the case, the spicules are still contaminated by minute particles of organic matter — dust, fibre, etc. — they can (if siliceous) be treated according to Swatman's method for

diatoms. Drain off as much water as possible from the phial and add $\frac{1}{2}$-1 cc of concentrated sulphuric acid. Allow to cool and then drop in a small crystal of potassium chlorate. When this has dissolved, place the phial in an inclined position in a small water bath and bring to the boil. Cool and wash as above. Carbon may be removed by putting the spicules in a porcelain crucible, heating gently until dry and then covering with a layer of potassium nitrate and carefully heating until the mass is fused. After cooling, dissolve the mass in water and wash the spicules as already described. It goes without saying that some of the reagents mentioned are highly corrosive and must be handled with the greatest care.

Sectioning a sea urchin spine

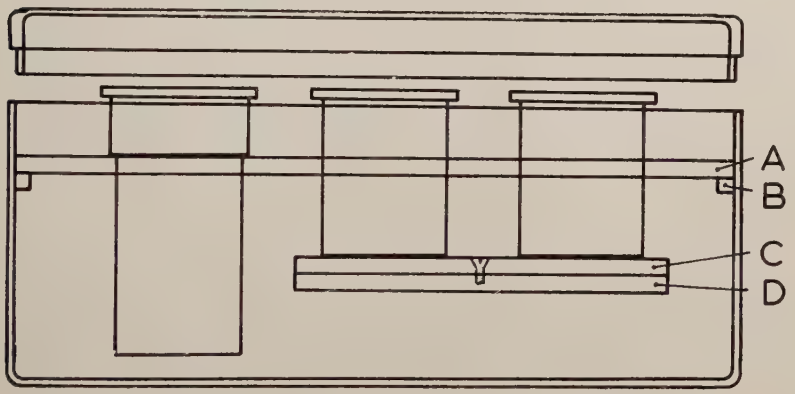
The spines of urchins found in warmer waters are composed of concentric rings of calcareous tissue reinforced at regular intervals by solid pillars of the same material. Sections of these spines are classical objects for the slide cabinet and are prepared by cutting and grinding in the same manner as sections of rock, but with certain additional precautions, on account of their nature. Cut the thinnest possible discs from the spines using a fine jewellers' or similar saw. These discs must not be so thin that they break up, and all dust be removed as far as possible to prevent clogging of the network. Rub one side of the sections gently on a fine flat file and then with water on a Water-of-Ayr stone. Wash, dry carefully first by blotting and then by gentle heat. Next the sections should be stuck to a glass slide, prepared faces down, using hardened balsam, re-heated on the glass until it is fluid so that it permeates all the interstices. The balsam should be hardened to the extent that when cold the mass can be just and only just, indented by the thumb nail. If it is harder it will be brittle, if softer it will allow the network to become clogged. The mass is next rubbed down as described above for the individual pieces until the sections have attained the correct degree of translucency. If all has proceeded correctly and the mass after cleaning up is found to be homogenous and the sections free from detritus, the coverglass may be applied, using liquid balsam and very gentle heat to avoid loosening the sections. It must be borne in mind that the ground surface of the sections will make them look far less transparent than they will be when finally mounted under the cover. If bubbles or other defects develop in the balsam, the sections must be freed by heat and re-mounted with care on another slide. When several sections are treated together they may not attain a uniform thickness unless each one has been pushed down into close contact with the glass. The only remedy is to detach the sections by heat and finish them on separate slides.

PLASTIC BOX FOR MICROSCOPE OPTICS

M. J. Hirons

A FITTED box on the bench to hold optical accessories while working with the microscope is most desirable. A Perspex or other transparent plastic container is admirable for this purpose: it is easy to clean, is dustproof if it has a fitted lid, and all the components are clearly visible.

Such a fitted box can be easily and cheaply made using one of the popular plastic sandwich boxes that may be purchased quite cheaply and which are available in a variety of sizes. One measuring approximately $5\frac{1}{2} \times 3 \times 2\frac{1}{2}$ inches is an ideal size to contain six eyepieces.



A: Shelf. B: Support. C: Lower Shelf. D: Support

The box illustrated has a shelf made from a sheet of $\frac{1}{8}$ inch Perspex, cut to fit exactly. The shelf is held in position by strips of $\frac{1}{8}$ inch square material cemented to the ends of the box. The eyepieces pass through holes cut in the shelf prior to assembly within the box. Eyepieces with a flange at the upper end stand above the shelf and are thus easily removed. Those having only a rim at the upper end may be supported in a convenient position by a second shelf placed about $\frac{3}{4}$ inch below the upper one. This second shelf is also supported by two $\frac{1}{8}$ inch square strips of Perspex, cemented in position or held by small retaining screws.

In my own box the shelves are removable so that they may be easily dusted. To retain the lower shelf in position I have inserted a small screw through the shelf and into the support.

FROM MY NOTEBOOK

D. S. SPENCE, M.B.

John H. Barton

Since the writer contributed the note about the Barton miscroscope, he has found out something more about its maker. John H. Barton worked with Ross and Co. until 1904, when he left to start an optical business in Clapham, continuing in existence until 1914 at least. This would probably be Barton, Linnard & Co., 196 Clapham Park Road, from which firm a letter about prism binoculars appeared in *The English Mechanic,* Vol. LXXXVII, page 419, 5th June, 1908. He later exhibited the 'Britannic Homogeneous-prism Binocular', described in *The Transactions of the Optical Society,* Vol. XXII, No. 2, but the writer has no further information about this, except that it was about 1921. He said he was designer in a firm which made scientific instruments, not microscopes.

Fluid Mounts

The writer was very interested in Mr. Phoenix's account of his experiments in *The Microscope,* Vol. 12, page 71. The writer himself made some experiments on similar lines some years ago, but, not having Mr. Phoenix's access to samples, did not get very far with them: so he hopes that Mr. Phoenix will persevere on his chosen line.

Fluid media may be divided into those which are stable, of which there are only three or four of the aqueous kind, and those which tend to evaporate, like dilute formalin. The only difficulty with the stable media is their possible chemical or physical reaction with the sealing substance. With the unstable media there is also the question of evaporation of water (or of some fluid chemical component of the medium) through the seal. There is no guarantee that a seal chemically stable to water will not allow water to evaporate through it. It seems to the writer that the running-in of varnishes of which Mr. Phoenix (in company with so many mounters in the past) complains is due simply to evaporation of something, presumably water, from the medium, so reducing its bulk and causing atmospheric pressure outside the mount to force the cement in. The writer has commented before on writers who have recommended putting an object in Canada balsam in xylene

in a cell, and covering at once. It should be remembered that most fluid media lose bulk in time.

Someone with some knowledge of physics will suggest, quite justifiably, that the water content of the air changes constantly, and that a fluid which is stable to a certain atmosphere will not be stable to one wetter or drier. This is quite true, of course: but the writer is of opinion that this effect may to a large extent be neglected. Let us consider a drop of glycerol sealed by a varnish, and then exposed to air which is not absolutely dry. The moisture of the air will pass through the seal (is 'invaporate' the word to described this ?) and the glycerol will tend to swell. If the reader can picture this as a disruptive force, he will probably find himself able to assume that, to a first approximation, the disruptive force is proportional to the difference between the concentration of the glycerol in the drop and the concentration of a drop of glycerol that would be stable to the surrounding air under consideration. (Mathematical students will be reminded of the preliminary assumptions in dealing with harmonic motion). The less the difference in concentrations, the less the tendency to swelling of the mount; and, of course, the same consideration applies in the opposite sense to a drop of dilute glycerol in a dry atmosphere. Now the actual concentration of glycerol in a stable solution is, it may be roughly assumed, about half way between that appropriate to dry air, and that appropriate to air of a damp climate; so that, for all ordinary purposes, the disruptive force will be far less than that sustained by a mount exposed to such extremes of dryness and dampness as never occur in ordinary circumstances.

Unfortunately there seems to be no cheap method of finding the water content of the air without taking a long time, so experiments on these lines are not likely to be done. One would like to expose mounts to controlled dampness.

Nitro-Alkydal varnish as mounting medium

Dr. Walter Hirling of Weil, Germany, has an article in *Mikrokosmos* for August, 1958 (Jahrgang 47, Heft 11, Seite 261) on this and gives it high praise for its clearing and lasting properties for mounting small insects, mosses, and such-like. It is a colourless varnish which sticks well to glass, and has been brought out by the firm of Bayer, Leverkusen, for the purpose of varnishing mounts of large objects in "Celodal."

Dr. Hirling says that, as long as the objects are fairly dry, no complete dehydration is necessary. Small air-bubbles soon disappear. Several photographs of insect and moss preparations illustrate his article. Do any readers know any more about the varnish ?

Far be it from the writer to have any objection to the introduction of new mounting media, but some considerations put

forward by his friend Mr. H. M. Hatherly come to his mind, and serve to moderate his enthusiasm. We know neither the composition of this substance, nor the method of making it; and the manufacturers may suddenly decide to change its composition or discontinue making it altogether, in which case the unfortunate microscopist would be left lamenting. If a compound is used for menapongolising the peladolators of rebinofoliasistors, and, after a lapse of time, the latter machines have to be scrapped owing to scientific advances, the compound will simply cease to be made, as the small amount used in microscopy would not suffice to pay the firm for its outlay. Much the same considerations deter one from many of the new mounting media advertised by dealers by names which mean nothing to the chemist.

Celodal

This, as the writer gathers from an article on it by Dr. Hirling in the next number of *Mikrokosmos*, page 286, is a water-soluble urea-formaldehyde condensation product, which may be used for mounting microscopical and larger preparations of animals and plants, and for infiltrating preparatory to using the microtome. It seems to be fairly popular in Germany.

'Methods of Analytical Histology and Histo-Chemistry'

This book, by Mr. Edward Gurr, is devoted to methods of identification of particles of various chemicals in histological sections, and will be very useful to workers in pathological and other laboratories. It gives full details of reagents and methods. Its price is £3 10s., and it is published in 1958 by Leonard Hill.

Nickel Acetate for preserving Chlorophyll

Mr. Erwin Beck, of Karlsruhe, has been working with nickel salts as fixers and for preserving the colour in green algae. He reports a good deal of success in *Mikrokosmos*, 47. Jahrgang, Heft 3, Seite 68, December 1957. He gives a table of the precipitation reactions of nickel acetate, which appears to be the most favourable, with various microscopical dyes. For fixing, he uses nickel acetate in water, in dilute acetic acid, in dilute formalin, and in a mixture of them. For algae he recommends fixing with nickel acetate 1·5 Grammes, Glacial acetic acid 1 G., and distilled water to 100 G., for six hours only, washing, and mounting in a mixture of nickel acetate, lacto-phenol, and glycerol-gelatine. Take ·4 G. Nickel acetate, 5 G. lactophenol and 95 G. water. Take 12 millilitres of this and mix with 34 ml. water, 7 G. Gelatine, and 45 G. Glycerol (Mr. Beck himself is responsible for this combination of weights and volumes, which the present writer finds rather unusual in a German scientific paper, though quite usual in

English writings). This apparently serves as mounting medium, and he says that the colour lasts very well; but he gives no actual mention of the time his slides have lasted. He recommends mounting in "Celodal" also.

Some of our readers may like to experiment on these lines. The writer cannot say how stiff a jelly results from the mixture; but of course one could seal it with soft paraffin. Mr. Beck gives no details of his actual mounting technique, nor, indeed, says whether he rings his mounts.

READING MICROSCOPICAL SOCIETY

FORTHCOMING MEETINGS:

8 February: Visit to the Veterinary Investigation Laboratory, Coley Park. Demonstration by R. M. Loosmore, M.R.C.V.S.
14 March: The Rhizopoda by W. Burrells.

Members are invited to a meeting of the Reading and District Natural History Society on 10th March — 'Stomata' by Professor O. V. S. Heath.

The Microscopical Society meets in the Microbiology Department, Reading University, at 7.15 p.m. Visitors are welcome. Honorary Secretary: R. E. Harper, 6 Fernbrook Road, Caversham, Reading.

THE DIATOMITE OF OAMARU (Vol. 12, No.1)

READERS are asked to note two corrections:

P. 3, line 17 and elsewhere, *read* 'Harry de Lautour'.

P. 3, line 29, *read* '. . . known as Borries) and Williams Bluff all followed . . .'

NEXT NUMBER

THE next number of THE MICROSCOPE will be published at the end of March. The contents of the issue will include —

How to Clean Diatoms.

Thoughts about 35 mm. Photomicrography.

Visibility and Resolution of Microscopical Detail: 6, Optical artifacts and spurious resolution.

Some Requirements for Matching Eyepieces for High Power Binocular Microscopes.

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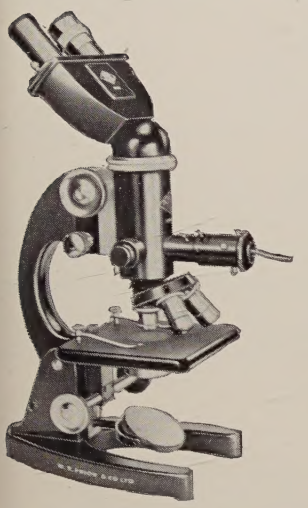

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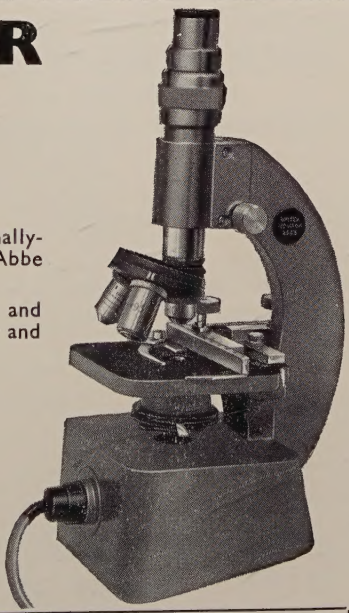
s can be seen from the illustration, a 1/12 in. oil mmersion objective and Mechanical Stage can be supplied as accessories.



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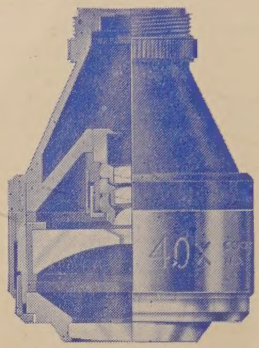

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